

Studies in Dedifferentiation.
II. Dedifferentiation and resorption in
Perophora.

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With Plates 26-28 and 1 Text-figure.

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1. INTRODUCTION.

THE observations of Driesch (1906) and E. Schultz (1907) and myself (unpublished) upon the reduction or dedifferentiation of the social Ascidian *Clavellina* have been mainly morphological. Accordingly I decided, while in the United States, to take up the problem from the physiological aspect. The work was carried out at Wood's Hole. *Clavellina* itself is not found there, but another social Ascidian, *Perophora viridis*, is common, and proved to be a useful form for experimental

work. As is well known, the social Ascidians reproduce asexually by means of buds given off at intervals from creeping branched stolons; but whilst in *Clavellina* the zooids may reach two inches, in *Perophora* the maximum length is only about one-quarter of an inch, and the span of life is probably limited in proportion. The branching and budding of *Perophora* is also much easier to follow, the stolons often growing in a straight line for a considerable distance, giving off buds at regular intervals. It is thus easy to trace a sequence from young to old individuals in *Perophora*, but hard in *Clavellina*. In *Perophora* it is also possible to isolate single zooids of any age by cutting the stolon midway between the neighbouring zooids on either side: and in such preparations the piece of stolon is of the same order of magnitude as the zooid, while in *Clavellina* the volume of the stolon is quite negligible in proportion to that of an adult, a half-grown, or even a quarter-grown zooid.

Such preparations we may call stolon-zooid systems. They are composed of two very distinct parts. The stolon is very simple: it consists of a thin external test-layer surrounding a single-layered tube of flattened ectodermal epithelium, which in its turn is divided into two by a horizontal partition composed of two very thin endodermic epithelia flattened together to form a single sheet; the space between ectoderm and endoderm contains blood, with numerous cells of several different kinds. At either end of the stolon the partition stops short, so that the blood can circulate from one half-tube to the other. It is normally kept in motion by the heart-beat of the zooid, which, as in all Ascidians, undergoes a periodic reversal of direction. The cut surface of test and ectoderm soon heals over. In a healed preparation the ectoderm at either (cut) end of the stolon is more or less cuboidal, and presents the appearance of undifferentiated tissue.

The zooid, on the other hand, is of high organization, containing, as it does, heart, stomach and intestine, elaborate branchial apparatus, nervous, muscular, and excretory systems, and hermaphrodite reproductive organs. It is also highly

sensitive in the region of the two siphons. It is connected with the stolon by a narrow tube of less diameter than the stolon, separating above into two tubes; this is generally longer in proportion in older individuals. The stolon may grow in length and form buds at the proximal end or the distal end or both.

Suitable food for *Perophora* has not yet been discovered; but in spite of this stolon-zoooid preparations may be kept alive in the laboratory for a considerable length of time.

2. DEDIFFERENTIATION.

(a) General.—Processes may occur in living matter whereby whole organisms or parts of them become visibly simpler. This occurs, for instance, in *Clavellina* when kept in unfavourable conditions, in *Hydra* when starved (Schultz, 1906), and in various other Coelenterates, in encysting protozoa and in other protozoa in the ordinary course of the life-cycle, without encystment (Lund, 1917), in sponges (Maas, 1910; Müller, 1911), &c. Such a process is the reverse of differentiation, and is best called dedifferentiation. It has also been termed involution and reduction. The latter word will here occasionally be used as a convenient synonym for the more accurate but clumsier term.

In *Clavellina* the original observations of Driesch and the later work of Schultz was carried out on half-animals, the individuals being cut in two and the half containing the branchial sac (pharynx) used for the experiments. This portion proved capable of regenerating the whole organism. Sometimes it remained intact and produced a restitution-bud in which the missing organs were formed; at other times it dedifferentiated completely to form an opaque spheroid which later redifferentiated into a normal whole individual; or it might show a combination of the two processes. Here, when dedifferentiation occurred, it was as the result of the shock of the operation and of the changes produced by it.

However, Driesch also mentions in one of his papers (1906) that he had been able to secure dedifferentiation in whole individuals. In my work I used whole individuals only. With them I found that the simplest method of obtaining dedifferentiation was to leave unchanged the water in which the organisms were kept, the accumulation of toxic waste products probably initiating the process. It was also found that only young individuals underwent dedifferentiation easily, mature and half-grown zooids speedily dying.

When full dedifferentiation, whether of half or whole zooids, occurs in *Clavellina*, a spheroidal white mass results, in which all the organs are very much simplified, both morphologically and histologically, becoming reduced to a series of separate sacs, some simple, others compound, of roughly spherical shape with walls of embryonic-looking cuboidal cells. On being replaced in clean water the opaque mass usually grows out to form a new perfect zooid, quite normal but smaller than the original; and this alternation of differentiation and dedifferentiation may be repeated several times. It is obvious that the term dedifferentiation may be applied equally to all retrogressive changes resulting in simplification of visible structure, provided that the reduced tissues remain alive, whether or no redifferentiation from the reduced condition is possible or not. When it is possible, an added interest attaches to the whole phenomenon; but dedifferentiation is essentially similar whether subsequent redifferentiation can occur or not, just as differentiation is essentially similar in all cases whether subsequent dedifferentiation can occur or not.

In *Perophora* similar methods were at first adopted, the animals being kept in watch-glasses containing approximately either 5 or 7.5 c.c. of water.

Zooids that were adult or more than half-grown never achieved successful reduction. They all died after a few days, but always after a preliminary attempt at dedifferentiation. The siphons were closed, all appearance of vigour and tone was lost, the body became contracted and opaque. The appearance

was very similar to that presented by an early stage of dedifferentiation. After this, however, a brownish colour appeared in the animals, and this heralded true degenerative changes leading to death. Adult individuals are often found in nature in a similar state, and these, too, always appear to die without full dedifferentiation; in fact it would appear that natural death occurs in *Perophora* through this means, the conditions in old zooids being such that they cannot maintain themselves in full tone, and thus undergo incipient dedifferentiation, which, in these old zooids, is not able to complete itself, and so leads on to degeneration and death. Similar failure of old individuals to adjust themselves to changed conditions is of course well known in the case of regeneration; a discussion of the whole subject will be found in Child's book, 'Senescence and Rejuvenescence' (1915*a*).

When smaller zooids were taken, however, quite different results were obtained.

(*b*) Simple Dedifferentiation (*Clavellina* type).

If the stolon be cut very close to the zooid on either side, the zooid will usually dedifferentiate as in *Clavellina*. That is to say, the siphons contract, the zooid shrinks, becomes increasingly opaque, and eventually draws right away from the tunic. The final stages of this process were represented by opaque spheroidal masses with a diameter of one-third to one-half that of the original zooid, and often with no or extremely slight trace of siphons. The heart usually continued to beat even in this condition. Examples are shown in fig. 1. Here the shortness of the stolon is noticeable.

In most examples of this process the stolon was either very short, or underwent dedifferentiation concomitantly with the zooid, or both. In all such cases the system, with its relatively small proportion of stolon, was similar to a stolon-zooid system in *Clavellina*, and behaved in an essentially similar way.

In one point there was a difference. I never observed such complete reduction in *Perophora* as in *Clavellina*. Further, I was not able to obtain redifferentiation by replacing the

spheroids in clean sea-water. This, however, is probably due simply to a greater susceptibility of *Perophora* to laboratory conditions, in the same way as one species may develop well after artificial insemination in the laboratory, while a closely-related species cannot be got beyond early segmentation stages.

(c) Dedifferentiation with Resorption.

(1) *Stolon Resorption*.—In systems with healthy young or moderate-sized zooids which were changed to fresh sea-water daily, the interesting fact soon came to light that so long as the full tone of the zooid was maintained and its siphons continued wide open, it did not decrease in size at all, but maintained itself at the expense of the stolon. This would also occur sometimes when the zooid was in the form of a partially-differentiated bud (e.g. fig. 4. *c-f*). The bud remained of the same size and at the same stage of development for over seven days, while the stolon was almost completely resorbed.

Later it was found that in other systems in which the zooid portion was represented by similar developing buds, these might not merely maintain themselves but actually develop further into perfect zooids at the expense of the stolon, e.g. fig. 2, where in the course of three days a very great change in the relation of zooid and stolon has taken place.

It is thus clear that in certain circumstances the zooid may be physiologically dominant over the stolon, and may either develop or maintain itself at the latter's expense.

(2) *Zooid Resorption*.—In other cases, however, a change in the opposite direction takes place. In most systems, after the lapse of a few days without change of water (and in some even when the water is changed), the premonitory signs of dedifferentiation become visible: the siphons close, the general tone decreases, and the whole animal shrinks slightly. But the sequel is quite different. Instead of becoming more and more opaque, on account of the cells of the various organs and epithelia becoming cuboidal and so bringing about a marked decrease in the size of all the cavities

in the organism, the zooid remains transparent. At the same time, however, it decreases in size. It is, in fact, being resorbed into the stolon. Appearances indicating the occurrence of this process are also found in nature, though not commonly. Successive stages of the process are shown in figs. 4, *a-b*, 5, 6, 9, 12, and isolated stages in figs. 7, 8, 10, 11, 13-15.

After a very short time the siphons disappear entirely, and a spheroidal mass of two-thirds or one-half the zooid's original diameter is left. In this, the ovoid heart, very little diminished in size, can always be seen pulsating steadily. A steady diminution of size continues, the heart too decreasing absolutely, although becoming relatively larger. A certain degree of opacity may appear, but it is never striking.

At a certain moment the pulsation of the heart slows down and ceases. Soon after this the heart becomes invisible altogether. Traces of other organs are visible. At first they are somewhat masked by the slight opacity caused by accumulation of blood-cells in the shrunken zooid, but later, as the zooid becomes smaller and smaller, they become increasingly clear. At about the stage when the heart disappears they are seen as two or three translucent rounded bodies, some colourless, some faintly yellowish.

The shrinkage continues after the disappearance of the heart, and soon the zooid comes to appear as a minute knob, scarcely bigger than the stalk connecting it with the stolon. This stalk represents the stolon-connexion of the original zooid, and has itself decreased in size, although but slightly. At this stage a single clear refractive area, which I take to be the vestige of the stomach, is usually the only structure to be seen in the knob. Finally the knob all but disappears, and a mere trace of the clear area remains visible. Presumably the stalk itself would also eventually become resorbed into the stolon, but resorption is much retarded after the cessation of the heart's action, and becomes progressively slower and slower as the size of the zooid decreases, so that I have never actually observed this ultimate step in the resorption of fully-formed

zooids. Complete resorption of very young buds has, however, been noted. When dedifferentiation is rapid, and especially in larger zooids, the connexion between zooid and stolon may be severed, and a spheroidal mass left isolated in the old tissue. This, of course, precludes further resorption.

Two further points of interest should be mentioned. The first is that the tunic of the zooid undergoes considerable decrease in size, presumably by means of some form of resorption. This reduction, as shown in the figures, is usually irregular, but I have seen cases of reduction in buds where the test remained closely apposed and of firm outline.

The second is that the stolon, especially during the late stages of the process, performs spontaneous movements of contraction, thereby causing a rudimentary and irregular form of circulation through the system. This may be called *stolon-circulation*. The contraction is effected by the ectoderm cells becoming cuboidal in one place and later extending again to become flattened 'pavement'-epithelium (fig. 24). Corresponding with these circulatory movements back and forth, the now minute zooid could be seen now to contract, now to expand slightly, cells moving from it into the stolon or vice versa. A similar contractibility of the ectoderm I have also observed in the stolon of *Clavellina*, and in the coenosare of Hydroids (*Campanularia* and *Obelia*).

During the resorption of the zooid the stolon usually grows in length, at least during the earlier stages (figs. 5, 6*a*). Later on the stolon often remains constant in size, or decreases slightly. It then becomes more or less opaque, owing to the accumulation in it of cells from the zooid. Such packed opaque stolons, however, may send out transparent slender new growths at one or both ends. Quite often the final length may be greater than the original length, and buds may even be formed. The process of resorption may take a considerable time. The zooid in fig. 9 took seven days in all, four days to the cessation of the heart-beat and three days more until only a stalk was left, but in other specimens it was much more rapid. For convenience the process may be divided into

stages as follows: (1) shrinkage alone, (2) siphons closed, (3) siphons withdrawn from test, (4) spheroidal form assumed, (5) cessation of heart-beat, (6) reduction to stalked knob.

It will be seen that this process is the reverse of that previously described as stolon-resorption. In both cases, however, the equilibrium of the stolon-zooid system is altered, the alteration results in the resorption of one or other of its members, and this resorption may be total.

Resorption of an organ like the stolon cannot be considered a very unusual phenomenon. It is paralleled, for instance, by the resorption of various larval organs at metamorphosis, such as the gills and tail of a frog-tadpole. Resorption of whole individual organisms, however, is much more unusual. So far as I am aware, it has only been noted at all adequately by Loeb (1900), who found it to occur in the Calyptoblast Hydroid *Campanularia*. I have re-investigated the phenomenon in *Campanularia* and also in *Obelia*, and can confirm the facts entirely. Something rather similar occurs in those Echinoderms where almost the whole of the larva is absorbed into the growing rudiment of the adult, but there remains an essential difference, namely, that resorption in such a case is determined as part of a normal development, whereas in *Perophora* and *Campanularia* it does not occur except as the result of circumstances which must be called abnormal. This is also true for the interesting observation made by Child (1904), who found in the chain-forming Turbellarian *Stenostomum* that, if a cut be made through one of the zooids, the posterior half of such a zooid is completely resorbed by the zooid behind it. Resorption of whole zooids is also recorded (see later, p. 675). The case of *Perophora* is more remarkable than any yet recorded, partly owing to zooids being resorbed by subordinate systems, and partly owing to the great complexity of the zooids, which is very much greater than in Hydroids or Turbellaria.

In all three cases, however—Ascidian, Flatworm, and Hydroid alike—the mechanism of resorption appears to be the same, namely, that the organs all decrease in bulk by the

actual migration of single cells out of their union in the tissues into the cavities of the body (in Hydroids into the coelenteron, in *Stenostomum* into the parenchyma, in *Perophora* into the haemocoel). In no other way can we explain the rapid decrease in size of the zooid, or the marked increase in the number of cells in the cavities. The stolon in *Perophora* always becomes crowded with cells during the later stages of resorption. I have seen no sign of the cells disintegrating on release, there being no increase in the number of granules, &c., in the plasma; and the process can certainly not be explained as due to the using up of cells as nutriment *in situ*.

We have thus the singular spectacle of the organs and tissues unbuilding themselves. It is as if a house were to become smaller and smaller through individual bricks leaving their places here and there in the walls and accumulating in the passages and garden, the rooms meanwhile closing the gaps in their walls and progressively diminishing in size.

During the process it appears that dedifferentiation also is going on. For one thing, the ectodermic epithelium becomes more and more cuboidal, and then also all cells that appear in the blood-stream are of a simple, irregularly-rounded type, and not visibly specialized in any way.

The long persistence of the heart as a functional organ, and its final sudden disappearance are closely paralleled in simple dedifferentiation in *Clavellina*.

Presumably what occurs when the stolon is resorbed into the zooid is similar, the cells of the ectodermic epithelium and of the endodermic partition also becoming dedifferentiated and migrating out of the tissues into the blood-stream. The process is merely not so remarkable here, owing to the less differentiation of the tissues involved, and the subordinate status of the stolon as an organ. To sum up, we find that in *Perophora* (and in *Campanularia*) adverse conditions lead to a form of reduction in which dedifferentiated cells migrate out of their fixed position in the tissues into the general cavity of the body, and the whole differentiated zooid finally disappears by resorption. This combination of dedifferentiation

and resorption will probably be found to occur also in other colonial organisms, the zooids of which are united by relatively undifferentiated portions.

When the stolon is resorbed in *Perophora* a similar process appears to be at work. It is further probable that in many other cases of resorption of subordinate organs, and of grafted tissues, a combination of dedifferentiation and resorption is also taking place, although in many higher organisms the factor of phagocytosis also enters, but probably often as a secondary phenomenon.

3. EXPERIMENTS WITH POTASSIUM CYANIDE.

The next step was to find out something as to the factors involved in the reversal of dominance and the initiation of resorption. With this end in view some experiments with dilute solutions of KCN were made. I have to thank Professor Child for advice.

As a preliminary the effect of an $n/250$ solution of KCN in sea-water was tested. It was found that this affected the whole system, zooid and stolon alike. Shrinkage of all parts took place, and death-changes were in progress after twenty-four hours. A series of solutions was therefore prepared as follows: $n/250$, $n/500$, $n/1,000$, and so on to $n/64,000$, together with a control vessel. All vessels were protected as far as possible from evaporation, and the solutions changed every twenty-four hours.

The detailed results are to be found in Table I. They may be summarized as follows: Solutions of $n/1,000$ and higher concentration affect both stolon and zooid very adversely, and lead to death in about forty-eight hours. The ciliary action of the gills is much slowed down, and the action of the heart badly affected. Almost always the stolons become contracted and opaque. The zooids were never drained completely by resorption; they usually shrank slightly, became opaque, and then died. In one or two cases the appearances were very similar to those seen in the dedifferentiation of *Clavellina*. In solutions from $n/2,000$ to $n/8,000$ inclusive

there was no growth of the stolons (except a very slight growth in one case). In $n/8,000$ the appearance of the stolons was nearly normal, but in the two higher concentrations they were adversely affected and showed contraction. As regards the zooids, the circulation was in all subnormal. A considerable degree of draining (resorption) took place, but was never complete. Several became opaque and spheroidal without appreciable draining (*Clavellina* type of dedifferentiation). The zooids mostly still showed normal tone after twenty-four hours, while in higher concentrations all had begun to shrink by this time. A slight effect on the stolon was indicated by opacity and clubbing of the ends.

In solutions from $n/16,000$ to $n/64,000$ inclusive, a considerable proportion of the stolons showed new growth. In no case was the stolon adversely affected, but it always remained of normal appearance with flat cells. Of those zooids which did not die the large majority had begun to be resorbed in the typical way before forty-eight hours, and some of them became completely drained. The $n/32,000$ solution seemed to be the most effective in causing this draining, but this may have been an accident, although it is perfectly possible that the $n/64,000$ solution is less effective because too weak.

The controls, apart from a small proportion which started to drain early (an occurrence which takes place in all collections of stolon-zooid systems chosen at random, and presumably depends on the internal condition of particular zooids), remained normal, the zooids completely expanded, for forty-eight hours and most of them for seventy-two hours. Most of them showed slight new growth of the stolons, as is customary in the early stages of stolon-zooid systems, but they were not kept long enough to see whether stolon-resorption, which only occurs after several days, would supervene.

We can classify the effects broadly as follows. High concentrations kill the whole organism speedily. The next lower degree of concentration causes contraction (dedifferentiation) of both stolon and zooid. No resorption is possible in this case, whether of the zooid or of the stolon. The next

lower grades of strength adversely affect the zooid, but only affect the stolon sufficiently to inhibit its growth, not to cause its dedifferentiation. Partial resorption may take place in these circumstances.

Still lower concentrations have no appreciable effect upon the stolon, but yet adversely influence the more sensitive zooid. The stolon is thus able not only to maintain its form, but to grow. The zooid starts dedifferentiation, and this is followed by resorption, which, typically, is complete. Finally, we get dilutions beyond which no effect is produced on the zooid or the stolon, with the result that the normal dominance of the zooid is maintained, and it is the stolon which is resorbed.

We thus see that these processes occurring in nature can be experimentally controlled to a considerable degree. Other toxic agencies were not tried on *Perophora*; but from what we know of the reactions of other organisms we should expect that the results of KCN treatment are non-specific, and that essentially the same phenomena would occur in other toxic solutions.

Our results of observation are therefore to be thought of as due to the following causes:

(1) In *Perophora*, in the absence of food, there is a competition for nutriment among the parts of the colony.

(2) In normal conditions, in the absence of food, the most active and differentiated parts (the zooids) are dominant in this competition over the less active and differentiated parts (the stolons), which are used up as nutriment by the zooids.

(3) Correlated with this difference of success in competition there is a difference of susceptibility, the more highly-organized zooids being more susceptible than the stolon to unfavourable agencies.

(4) The result of unfavourable agencies on *Perophora* is to cause dedifferentiation.

(5) Once dedifferentiation has started the zooid ceases to be more active than the stolon, and so ceases to be dominant in the intra-organismal struggle.

(6) In *Perophora* dedifferentiation may be followed by

resorption due to the migration of cells from the tissues into the blood-stream ; when the stolon is little affected, therefore, zooid-resorption, or the reverse of (2), occurs.

In the most general terms we have a system the two parts of which are in equilibrium. This equilibrium may alter in either of two opposed directions. There is differential activity of the two parts ; the one which is more active is capable of causing the reduction of the other and utilizing it as food. But differential activity is correlated with differential susceptibility, which results, in certain unfavourable conditions, in a reversal of the direction of change ; for these induce dedifferentiation of the zooid, and in this condition it is less active than the stolon.

Similar conditions, viz. (1) a balance in an organic system ; (2) differential activity of the parts of the system leading to physiological dominance of the most active part ; (3) consequent differential susceptibility of the parts leading to a possible reversal of dominance ; and (4) the resultant reversibility of the reactions of the system—play an important part in general physiology. Often they are not easy to investigate ; but in *Perophora* we are fortunately provided with an organism in which they appear in a striking form, and are readily accessible to study.

It should be added that in all but the weakest KCN solutions a grey tinge, not seen in dedifferentiating individuals in sea-water, was observed in the zooids during resorption.

4. EXPERIMENTS ON REDUCTION IN ANIMALS WITHOUT CIRCULATION.

At Professor Loeb's suggestion, to whom I here tender my thanks, experiments were undertaken to see whether the action of the heart in *Perophora* was stopped by potassium chloride, and if so whether zooids without an active circulation would show typical reduction.

The experiment was carried out as follows. A large and a small stolon-zooid system were placed together in finger-bowl

TABLE I

EXPERIMENTS WITH KCN.

Series A. Young and medium individuals.

Series B. Very young individuals and almost complete buds.

A and B, four stolon-zooid systems in each vessel.

Dediff. = opaque, Clavellina type of reduction. Stages 1-6 refer to stages of resorption.

<i>Strength of KCN.</i>	<i>Twenty-four hours.</i>	<i>Forty-eight hours.</i>	<i>Seventy-two hours.</i>	<i>Remarks.</i>
Control A	Normal	Normal	3 normal, 1 draining, stage 3	New growth on all stolons.
Control B	3 normal, 1 dediff.	2 normal, 1 dediff., 1 draining, stage 4	As at 48 hrs.	Slight new growth on all stolons.
n/64,000, A	3 normal, 1 stage 4	2 dead, 2 stage 5	—	Stolons healthy, new growth on 2.
n/32,000, A	Normal	All unhealthy, 1 stage 4	—	Stolons healthy, slightly turgid, new growth on 2.
n/32,000, B	2 normal, 2 stage 3	All draining, stages 3-5	2 dead, 1 stage 5, 1 stage 6	Stolons healthy, no new growth.
n/16,000, A	3 normal, 1 dediff.	2 dead, 1 stage 5, 1 stage 3	—	Stolons healthy, new growth on 1.
n/16,000, B	2 normal, 1 stage 3, 1 stage 4	1 dead, 1 dediff., 2 stages 5-6	—	Stolons healthy, new growth on 2.
n/8,000, A	Expanded, circulation affected	1 dead, 3 dediff.	—	Stolons nearly normal, no new growth.
n/8,000, B	2 normal, 2 stage 3	1 stage 1, 1 dediff., 2 stages 4-5	1 dead, 1 dediff., 2 stage 5	Some stolons healthy, some opaque, 1 with new growth (very slight).
n/4,000, A	Expanded, circulation poor.	2 dying, 2 stage 4	—	Most stolons contracted; no new growth.
n/4,000, B	2 normal, 2 stage 3	1 dediff., 3 stages 4-5	All dead	All stolons opaque; no new growth.
n/2,000, A	Expanded, circulation very poor	1 dying, 2 dediff., 1 stage 4	—	All stolons contracted. (Cilia slow.)
n/2,000, B	2 subnormal, 1 stage 3, 1 stage 5	2 dying, 1 dediff., 1 stage 6	—	All stolons opaque, clubbed.
n/1,000, A	All subnormal	All dead or dying	—	All stolons early affected.
n/500, A	1 subnormal, 3 dediff.	All dead or dying	—	All stolons early affected.
n/250, A	All abnormal, dediff.	2 dead, 1 dying, 1 dediff., un-healthy.	—	All stolons early affected.

containing 50 c.c. sea-water together with a certain amount of $n/2$ KCl. The results are summarized in Table II.

TABLE II

+ denotes active heart-beat; (+) slow; (-) slow and intermittent; - no heart-beat. The upper sign in each compartment denotes the larger zooid, the lower the smaller.

No. of c.c. $n/2$ KCl added.	Minutes.								
	15	20	30	35	40	50	70	160	
0 (control)	+	+	+	+	+	+	+	+	+
	+	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+	-
	+	+	+	+	+	+	+	+	-
4	+	+	+	+	(+)	-	-	-	-
	+	+	+	(-)	-	-	-	-	-
8	+	+	+	+	not	-	-	-	-
	+	+	+	(-)	noted	-	-	-	-
10	+	+	+	(-)	-	-	-	-	-
	+	+	+	(-)	-	-	-	-	-
15	+	+	+	+	(+)	-	-	-	-
	+	+	(-)	(-)	(-)	-	-	-	-
20	+	(-)	-	-	-	-	-	-	-
	+	(-)	-	-	-	-	-	-	-
40	+	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-

KCl thus exercises a very marked effect upon the Ascidian heart. The stronger action of the salt on small zooids is to be noted. The organisms were left in the solutions to see what type, if any, of dedifferentiation they showed.

Those in the two highest concentrations died in under twenty-four hours without reduction; their stolons also were killed or damaged. Those to which 10 and 15 c.c. KCl had been added were scarcely affected after twenty-four hours, but were dead by forty-eight hours, having previously shrunk very considerably and become opaque.

In the solution with 8 c.c. one had died; the other had started to dedifferentiate. Both stolon and zooid were affected (fig. 20). The zooid showed a characteristic sign of KCl reduction in the cellular strands extending from the retracted siphons to the test. Also characteristic, and directly dependent on the absence of circulation, was the congestion

of the network of small blood-vessels close to the surface with the green blood-corpuscles. This gives a premature green opacity to zooids dedifferentiating in KCl. This animal was dead on the succeeding day.

In the solution with 4 c.c. one died, after only slight reduction, after three days. The other exhibited dedifferentiation of a type very similar to that just considered, but this time accompanied by a little growth in the stolon, which remained healthy and tonic. Although reduction had started, resorption never ensued, and after five days the zooid had died and was represented by a blackish spheroidal mass about half its original diameter, while the stolon was still healthy. (Fig. 19.)

In the solution with 2 c.c. matters were very similar. The stolons remained healthy, though distended with blood-cells (and possibly others) from the zooids, for over five days. The zooids withdrew their siphons from the test, shrank, and became opaque (i.e. started to dedifferentiate), but died with change of colour to brown or blackish before any marked resorption had occurred.

It will thus be evident that there are at least two factors concerned in resorption in *Perophora*. The first is the shrinkage of the whole organism and reversion of its cells to a cuboidal type which we may call simple dedifferentiation, the second is the migration of cells out of the tissues, which does not take place, or takes place only to a negligible degree, in the absence of the circulation.

Thus in the presence of KCl, with consequent cessation of heart-beat, the aspect of the process is altered in many particulars. High concentrations of KCl damage both zooids and stolon, and both contract. The cessation of the circulation in lower concentrations leads to a very speedy dedifferentiation of the zooid; but this never goes very far before death supervenes, and is unaccompanied by resorption.

The experiments were repeated, with variations, with forty-five more specimens; essentially similar results were obtained. Twenty of these showed dedifferentiation without resorption. In addition one showed a slight, one a moderate,

degree of resorption. Twelve formed new stolon outgrowths of fair length. The solutions used were 2 c.c. and 4 c.c. $n/2$ KCl in 50 c.c. sea-water.

5. EXPERIMENTS WITH LOW TEMPERATURE.

Eight vessels, each containing several individuals, were put in an ice-chest, with a temperature of 3° to 8° C.

Several points were noted when these were examined eight days later. Over half had turned brown or blackish, and were dead or dying. No cases of extreme or even considerable resorption were found. Most healthy-looking individuals had shrunk and become opaque, i.e. had dedifferentiated. The opacity was more marked than usual. Usually, however, the siphons were left open and attached to the test at a stage when at room-temperature they would have been closed and withdrawn. The heart-beat was very slow or absent, though the heart was usually visible. Sometimes the heart-beat began again soon after transference to room-temperature for examination. Very young individuals were less dedifferentiated than older ones.

The stolon seemed to be unaffected, and often remained of normal appearance even when the zooid was dead or dying; no new growth, however, was ever seen. Recovery did not occur at room-temperature.

Here again it is clear that the zooid has been much more affected than the stolon, and that the slowing or cessation of circulation has, as in KCl, prevented resorption.

In one system a new bud was produced on return to room-temperature, and grew to a normal zooid after six days.

6. MISCELLANEOUS NOTES.

(a) *Tone of Stolon.*—The turgescence of the stolon appears to depend on two quite different causes—first the physiological condition of the ectoderm cells, and secondly the pressure of the blood. Observation on a stolon which was undergoing retraction showed that the ectoderm cells were capable of great passive extension. At intervals the tip of

the stolon was dilated by the blood-pressure, the flattened ectoderm cells becoming still more flattened. The test also underwent passive dilatation.

However, even when the heart has ceased to beat, the stolon may be quite turgescient, and the ectoderm cells flattened, not cuboidal. Fullest turgescence, however, is thus only to be expected when the circulation is active and when the ectoderm cells are healthy.

It may be mentioned that the first step in dedifferentiation may be regarded usually as a diminution of tone (turgescence).

(b) Growing-points of Stolon.—At the tips of growing stolons the ectoderm is usually columnar (fig. 26) and the lumen generally filled with a dense mass of cells, into which the circulation does not penetrate. Sometimes, as in fig. 27, there is an increase in the number of green cells as we pass away from the tip. Often a layer of blood-cells will become attached to the walls of the stolon over a considerable distance, giving it an opaque appearance, though circulation continues internally.

(c) Lateral Outgrowths of Stolon.—Some lateral outgrowths, as in fig. 25, were occasionally seen. They did not represent rudimentary branches. Their meaning and origin is obscure.

(d) Attachment of Stolons.—The stolons will usually attach themselves to the substratum. This I have seen accomplished within three and a half hours.

(e) Bud-formation.—When medium-sized zooids attached to stolons of fairly large size were employed, buds were often formed from the stolon when dedifferentiation began in the zooid. Sometimes two buds or more might form. Buds may form at either or both ends of a piece of stolon. Resorption might occur at any stage in the development of the zooid from the earliest bud up to half-grown individuals.

(f) Penetration of Zooids by Stolon Branches.—An individual was seen in which apparently a branch of the stolon had grown up inside the test of the stolon-connexion and

encircled the zooid. The actual origin of the branch could not be traced *in vivo*. When old zooids die, stolon branches will frequently grow into the test previously occupied by the zooid.

(g) *Death-changes*.—Death-changes in *Perophora* usually involve a change of the green colour to a hard brown or black.

(h) *Change of Position of Stolon*.—When a stolon-zooid system is isolated, and new growth of the stolon with subsequent bud-formation takes place at one end, not only may the original zooid be completely resorbed, but the stolon tissue may abandon the original region and become concentrated in the region of the new bud. This ‘moving-on’ of the stolon is common in regeneration in Hydroids.

(i) *Segmentation of Stolon*.—In not very dilute solutions of KCl and KCN in which the stolons were affected, the stolon-tissue sometimes contracted into a series of separate ellipsoid portions giving the appearance of a necklace without a string.

7. EXPERIMENTS ON OTHER SPECIES.

(a) *On Amaroucium*.—Some experiments were also made on a form of compound Ascidian very abundant at Wood’s Hole—*Amaroucium pellucidum*, var. *constellatum*. For information and advice as to this form I have to thank Professor Caswell Grave.

Twenty small pieces of *Amaroucium* colonies, consisting each of from two to twelve or fifteen individuals, were cut out and placed in separate dishes in a small volume of water. The experiment was started on July 11 and was terminated after twenty-nine days. Controls were kept in the circulation-tanks.

Those kept in the unchanged small volumes of water showed alterations as follows. The larger pieces remained normal longer than the smaller. The larger individuals, however, usually showed reductional changes sooner than the smaller, *ceteris paribus*; but they did not usually remain as healthy as the small ones during reduction. Often they

exhibited a phenomenon characteristic of *Amaroucium*—the protrusion of the pharynx from the test and its subsequent decay, the abdomen and post-abdomen remaining and dedifferentiating. The small individuals underwent a process obviously analogous to the dedifferentiation of *Clavellina*. They shrank in size and decreased in transparency. The siphons at first remained attached to the test (unlike *Clavellina*), but later became completely detached. The pharyngeal region, as in all other reducing *Ascidians*, shrank much more than the rest, and finally a stage was reached in which the two main portions of the body were still distinguishable, separated by a slight constriction; the general shape was thus that of a constricted sausage; the organism was completely opaque, the colour being white with patches of red. (Certain organs of the normal zooid show this same red colour.) A curious feature was the frequent formation of clear projections of the test. These were generally stalked, and spheroidal or ellipsoidal, like bubbles or bladders. Healthy-looking test-cells could be seen in them. Very frequently new buds would be formed from the dedifferentiating zooids during the process of reduction. These would attain a certain degree of organization, but would not usually reach full development unless the piece were replaced in clean and regularly-changed water. This replacement in clean water, however, did not lead to the redifferentiation of the reduced original zooids.

After seven to twenty days, when it had become evident that it was not possible to obtain the extreme stages of dedifferentiation seen in *Clavellina*, the surviving pieces were all placed under gauze in the circulation. When examined twenty-nine days after the inception of the experiment it was found that a few had remained in approximately the same condition in which they had been placed in the circulation. More than half, however, while the original zooids had not redifferentiated, had given rise to new zooids, usually in one or two clusters of four to six zooids each.

It thus becomes clear that *Amaroucium* shows yet a third type of dedifferentiation. The specialized method of forming

a large number of buds practically simultaneously by segmentation of the very long post-abdomen, with subsequent differentiation of each segment to form a whole zooid, is apparently responsible for this. After dedifferentiation of the primary zooid has proceeded a certain way, either death supervenes or else the post-abdomen, released from subordination now that the dominant region is thus adversely affected, manifests its independence by producing new individuals. Once these new individuals start to develop they become dominant. The non-recovery of the partially-dedifferentiated original zooids may be ascribed to this, or to greater susceptibility. In spite of this absence of the power to redifferentiate the process of dedifferentiation is very similar to the early stages of the same process in *Clavellina*. For such behaviour there is ample evidence as regards numerous forms reproducing asexually in the work of Child and his pupils (Child, 1915*b*). We may thus say that, under the conditions which prevail in the colony, or in pieces of it, in *Amaroucium*, complete dedifferentiation of single zooids is not possible. The colony or piece regarded as a whole, however, may be said to undergo dedifferentiation followed by redifferentiation.

Oozoites.—These had the advantage over blastozoites that they could be obtained singly. They were got by allowing larvae to metamorphose in the laboratory. They could be induced to dedifferentiate either by lack of change of water, or, after a longer period, by starvation. The process was very similar to that in the blastozoites, with the exception that the formation of buds was never observed. This latter fact is undoubtedly to be correlated with the small relative size of the post-abdomen and the small absolute size of the whole organism.

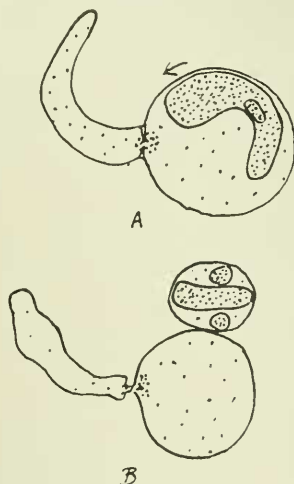
Here, too, dedifferentiation never got beyond a stage in which a sausage-shape was assumed (fig. 27, Text-fig. 1). The complete opacity and the spheroidal shape of the final stages of the process in *Clavellina* were not observed; neither did I succeed in obtaining redifferentiation.

On the whole, dedifferentiation in oozoites went a little further

than in blastozoites, and appeared to be a healthier process unaccompanied by so many abnormal swellings of the test, extrusions of parts of zooids, phenomena of local decay, &c.

Treatment with Alcohol.—A few experiments were made to test the effect of a 2 per cent. solution of alcohol on the process. It appeared that under its influence, dedifferentiation, both in oozoites and blastozoites, started sooner

TEXT-FIG. 1.



Reduction in oozites of *Amaroucium*.

- A. Zooid in stage 3, test spherical, test of tail degenerating.
 B. As A, except that the zooid shows detached cell-masses, and lies in a spherical portion of test detached from the rest.

than in the controls, but that it did not progress in a normal way. Opacity might be attained, but the loss of form, especially in the pharynx, was not as great as usual, e.g. the siphons remained visible relatively much longer after their retraction from the test than in normally-reducing specimens (fig. 28).

This appears to indicate that there are two distinct processes at work in normal dedifferentiation, the first being a mere shrinking as a result of exposure to an unfavourable environment, the second a real despecialization of the cells, resulting in loss of typical form. This latter then is due to active positive

changes in the cells, changes which are partially restrained by the action of a narcotic like alcohol.

It should perhaps be mentioned that not only oozites which had lived some time in the circulation, but also those which had only just metamorphosed, could be induced to dedifferentiate. Larvae were allowed to fix on slides on July 28. After seven to nine days in the laboratory they showed the first signs of reduction. On the tenth day their water was changed, but without effect on the result, for on the eleventh and twelfth days all were markedly reduced. A sausage-shaped mass, sometimes showing a slight constriction between pharynx and abdomen, lay in a much-swollen, but healthy, test, which was usually attached to the substratum in the form of a flattened sphere. The remains of the test of the larval tail could be seen attached to one point of the main test. In some examples an interesting modification was observed—a small portion of test surrounding the reduced zooid became constricted off from the main portion, which, though thus empty, remained healthy (Text-fig. 1, B).

In one or two specimens, detached, rounded masses of cells were to be seen outside the limits of the reduced zooid; these were also occasionally seen in reduced blastozoites. I believe them to have been derived from the organism itself, and not to have been merely collections of cells of the test. Such collections were also seen, but never had the compact appearance of the first-mentioned masses.

Swellings of the Test.—These have been already referred to. In connexion with experiments on dissociated sponges which were proceeding at the same time, it was decided to see whether portions of test were capable of re-organization or of regeneration in sea-water, or of growth in a nutrient solution. Accordingly a number of these 'test-bladders' were snipped off and isolated. After cutting the pieces were always torn and quite flabby. Some were placed in sea-water, others in weak solutions of peptone made up either in tap- or sea-water. In all cases a marked reorganization had taken place within twenty-four hours. The wound was completely

healed, and the piece was irregularly lobed, as if swollen out from two or more centres. It would appear that there was an actual accumulation of fluid in the interior, as in the spheres produced by sponge choanocytes (Huxley, 1921*a*). Usually the test-cells were nearly absent in some regions, rather densely aggregated in others. No regeneration, however, took place, and death occurred quicker in the peptone than in the sea-water. Death took place in one to three days in peptone, two to four in water. An interesting point was that, before death, many of the test-cells always left the matrix of the test, and crawled out on to the bottom of the dish. They still preserved their characteristic shapes at first, but eventually all rounded off preparatory to dying.

(*b*) *Botryllus*.—This genus is unsuitable for experiment owing to the small size of its zooids and their intimate connexion. One system, however, was seen in which all the zooids had become reduced to shapeless but healthy-looking lumps; the test round them had degenerated save for a thin layer. Some form of dedifferentiation had obviously occurred.

8. DISCUSSION.

Perophora happens to be an organism in which dedifferentiation and resorption affect the whole individual in a very striking way. In higher forms, thanks to their self-regulating mechanisms, their size, the bulk of their skeletons, and other factors, the processes do not affect the individual as a whole. None the less, similar processes play a large part in many phenomena, both normal and abnormal, throughout the animal kingdom.

In the first place it is important to realize that the 'struggle of the parts', to which Roux (1881) first drew attention, is a very real struggle; that the organism is in one aspect simply an equilibrium between a number of parts, some in a relation of simple competition, some in a relation of control or subordination to others; and that the relative success or failure of any one part, the degree to which it is developed, depend or have depended upon its success in this struggle.

Secondly, we must realize that success in the struggle, i.e. time and degree of development, may depend largely on rate of metabolic activity. It is not for a moment suggested that this is the only factor at work, nor that it is the most important factor (in the higher organisms the relationship of the nervous system to the tissues of course masks it to a considerable extent), but that it is an important factor.

Child (1915 *b*) has drawn attention to its importance for problems of regeneration and asexual reproduction; he finds that the most actively-working portion of the organism (or, in higher forms, the portion containing the higher centres of the nervous system) is not only formed first in regeneration, but exerts some sort of controlling effect upon the rest of the organization of the body. For instance, once a head is formed in the regeneration of a Planarian or an Oligochete the old organs are remodelled, some being broken down, others built up, until what exists stands in normal relation to the new head. But if, for some reason or other, a head is not formed (in Planaria it can be experimentally prevented from forming), then this remodelling does not occur. The production of a new pharynx, for instance, in a pharynxless posterior half of a Planarian, will not take place unless a head is formed at the anterior end.

However, this controlling effect of the head is only exerted up to a certain distance. Once this distance is overpast the tissues of the body are free to react in the way characteristic for them when not under any control, i.e. by the formation of a new head. In other words this control or dominance of the head or oral end (or apical bud in plants) is what regulates the important temporal and spatial relations of asexual reproduction. As is to be expected, it varies with external circumstances, and Child has performed some pretty experiments on the experimental control of dominance.

It would appear, especially from some of his recent work upon plants, that this dominance exerts an effect analogous to that of the nervous system by means of some form of conduction, and that it is not, as might at first be expected,

simply dependent on nutritional relations. Child has naturally stressed this important point (Child, 1919). However, the nutritional aspect is also important, and does as a matter of fact determine many relations of dominance and subordination of parts in organisms; and it is to some of the implications of this aspect that I wish to draw attention.

If one reaction or associated set of reactions is proceeding faster than another, in the same system, it will occur to a correspondingly greater extent; cf. Mellor, 'Chemical Statics and Dynamics', p. 70: 'In any system of parallel chemical reactions which consume the same substrate and are proceeding simultaneously in a mixture, the extent to which each reaction will occur is proportional to its velocity.' This means that if two sets of reactions are going on in an organism at an equal rate and that subsequently one of them is stimulated to a 10 per cent. increase, then the end-products of those reactions (the amounts of two different types of tissues, let us say) will, if the available food remains constant, change from the proportion 1000:1000 to 1048:952. A similar result will occur if the other reaction's intensity is correspondingly lowered. This is important in explaining many changes resulting from a change in environment acting upon the tissues which respond to the change at different rates (see Child, 1916; Robertson and Ray, 1920; Lillie and Knowlton, 1902, &c.).¹

One of the best examples is the relation of head-size to body-size in a regenerating piece of *Planaria*. Apparently the temperature-coefficient of the processes of the head-region is greater than those of the body, for the relative development of head increases with temperature. It is also decreased by increase in concentration of narcotics.

There is, however, another aspect of the question which it is rather more difficult to understand. That is the fact that if in an organism two sets of reactions are going on at different

¹ This will, of course, only occur up to a certain limit. A condition of hyper-activity may be induced, as for instance by excess of thyroid-secretion, or by excess of nervous stimulation in certain forms of neurasthenia, which results in a wasting of the tissues concerned.

rates, in two different regions, then, if the food-supply is reduced, the one which, *ceteris paribus*, has the higher speed will be able to maintain itself in its normal state and at its normal level.¹ This may be due to the fact that the assimilatory processes are reversible; this would imply that not merely the dissolved food-substances in the body-fluid are to be regarded as the 'substrate' from which the various reactions draw their materials, but that this substrate must be taken as including the tissues themselves. If, therefore, two reversible reactions A and B were proceeding simultaneously in two regions of an organism while the organism was starved, we should have each reaction making demands upon the end-products of the other, i.e. upon the tissues of the two regions. Four processes would therefore be involved—first and secondly, the reactions A and B proceeding in their normal direction; thirdly, B proceeding in reversed direction in response to the demands of A; and fourthly, A proceeding in reverse direction in response to the demands of B. Since A's speed is greater than B's, the end-product of A will continue to increase, while that of B progressively diminishes. We can represent such a state of affairs symbolically thus: $P \rightleftharpoons X \rightleftharpoons Q$, where P is the end-product of A, Q of B, and X the common substances utilized by both. If the rate of formation of P is greater than that of Q, the reaction will proceed until no Q remains.²

However that may be, we are confronted with the fact that if two reaction-systems are competing in the organism for an amount of nutriment which is not sufficient for both, then the more rapid, or the one which subserves the more highly-differentiated region, will not only get first call on the available nutriment, but will actually nourish itself at the expense of the other.

¹ This again is masked in higher animals by the fact that the nervous system, apparently owing to its controlling and co-ordinating function, has come to be the system least affected by starvation.

² Similar ideas are put forward by Runnström (1917) in his important paper on dedifferentiation in Echinoid larvae, to which unfortunately (owing to the war) I have only just had access.

This is particularly well seen in malignant tumours, which will continue to grow at the expense of the rest of the body, even when this is in a condition of relative starvation. In tumours derived from adipose tissue, the tumour-cells may be full of fat after all vestige of fat has disappeared from the normal tissues. The fact that regeneration will proceed actively and normally in starving Planarians exemplifies the same state of affairs.

We saw above that we should expect the reaction to proceed to a limit, all the product of the slower process being utilized by the faster. As a matter of fact this limit can often not be reached, since life is not possible when the 'subordinate' region is absent, or else its reduction brings about subsidiary changes.

It is also complicated by the supervening of dedifferentiation. That starvation can produce dedifferentiation has been shown by Schultz (1906), by Runnström (1917), &c. It therefore follows that the tissues of the less active region will usually, as a result of the starvation induced, reach a stage at which they are unable to maintain themselves, and will start to dedifferentiate. In the dedifferentiated state they will possess a still lower rate of metabolic activity, and so the resorption-process will be accentuated.

Next we meet with the fact of differential susceptibility. This is a corollary of difference in rates of reaction. The more highly-differentiated region and system, or the one with higher metabolism, will be, *ceteris paribus*, more susceptible to unfavourable conditions. If it is placed in a toxic solution, for instance, it will enter into reaction with more of it in a given time than will a slower system. There are a number of complicating factors (such as acclimatization) which enter into the problem, but, broadly speaking, we may say that a more highly-differentiated and more active system will be relatively more interfered with than a less highly-differentiated and less active system.

After a certain point of interference is reached, dedifferentiation will set in. Dedifferentiation is the primitive reaction of organisms to unfavourable circumstances. More energy is

necessary to maintain a cell in a differentiated than in a dedifferentiated condition. This is especially clear when, as in most instances, differentiation involves an increase in the surface of the cell relative to its bulk; here the maintenance of differentiated form alone involves the expenditure of more energy. Thus when the processes of life are interfered with by unfavourable agencies, the cell is unable to continue to produce the energy necessary for the maintenance of its differentiated state, and must either die or dedifferentiate.

The main characteristics of dedifferentiation are the following:

(a) Cells revert, if isolated to a spheroidal, if in epithelia to a cuboidal form.

(b) Cytoplasmic differentiation is lost.

(c) Organs containing cavities revert to simple spheroidal sacs. Junctions between organs are often broken.

(d) Apertures usually disappear altogether.

(e) The whole organism diminishes in size and reverts to a spheroidal form, owing to the form-changes in its constituent cells. This has the effect of increasing the opacity and density of the organism.

Once dedifferentiation has started in any region or system the previous level of metabolic activity in that system is inevitably much reduced. Thus, if dedifferentiation occurs in a dominant and not in a subordinate system, this dominant system will lose its dominance and become subordinate. Such alteration of equilibrium by unfavourable agencies we may call differential inhibition: it is a corollary of differential susceptibility. Differential inhibition need not, however, involve dedifferentiation, nor reversal of dominance. In a growing organism unfavourable agencies will depress the growth of the dominant or more active regions relatively more than that of the rest, and we shall, as outlined above (p. 669), get a decrease in size of the former, an increase in the latter—a decrease and an increase which will be absolute as well as relative. This is illustrated in some of Child's experiments (see later).

A chemical analogy, for which I am indebted to Mr. H. R. Raikes, of Exeter College, Oxford, may help illuminate the point. If one equivalent each of hydrochloric acid, boric acid, and ammonia are mixed, a negligible amount of boric acid will react with the ammonia owing to its small degree of dissociation. We may say that the hydrochloric acid is completely 'dominant' in the system, owing to a greater speed of reaction. If, however, the mixture is heated, the more volatile hydrochloric acid will be driven off, and the less volatile boric acid left to react with the ammonia. This we may call 'differential susceptibility' (to rise of temperature) involving 'differential inhibition' of one portion of the system, and consequent 'reversal of dominance'. If the mixture were contained in a very large closed space, cooling after heating would restore the original 'dominance' of the hydrochloric acid, giving a parallel to reversible dedifferentiation.

The emergence of the cells from the tissues in dedifferentiation is a phenomenon which deserves further study. Though probably by no means universal it is doubtless commoner than is generally assumed. It occurs not only in *Perophora* but also in *Hydroids*, in *Turbellarians*, and in *Echinoderm* larvae, and in many cases of actual poisoning, e. g. by mercury salts (Child 1917, Huxley 1921*b*) and other agencies (Gray 1920).

Once the cells start to emerge they may collect close to their place of origin, or if space and means of transport are available, be removed to regions at a distance. When the stolon portion is large in a *Perophora* stolon-zooid system, and the heart is beating normally, the latter is the case; it is also the case in *Hydroids* when the coenosarcial portion is large in comparison with the hydranth. The difference between the two possibilities appears to be similar to that between a reversible chemical reaction when the end-products are not removed, and the same when they are removed. Why, in the first case, the tissues should not simply resolve themselves into their constituent cells *in situ* is difficult to see, but the fact remains that they do not (e. g. *Clavellina*; *Perophora* with very small stolon attached, or with circulation stopped by KCl).

In ordinary organic systems, therefore, we must recognize that we may have to deal with any of the following phenomena :

(1) Physiological dominance and subordination of parts, manifesting itself first as regards conduction and control of asexual reproduction, secondly as regards nutrition.

(2) Differential susceptibility.

(3) Dedifferentiation.

(4) Differential inhibition.

(5) Resorption.

(6) Reversal of dominance.

Parallel phenomena occur in other organic systems in which parts are related in equilibrium. Thus dominance, subordination, differential susceptibility and inhibition, a form of dedifferentiation, and reversal of dominance, also occur in psychophysical systems, both in some where consciousness is involved and in some where it is not, as will be dealt with more fully later. Here the dominance may be called neurological and psychological, and the dedifferentiation is of course unaccompanied by physical dedifferentiation of nerve-tissues.

Many of the phenomena of inhibition, e. g. of buds by growing tips in plants, and within the central nervous system, obviously depend upon relations of dominance and subordination. A few examples will perhaps serve to illustrate some of these general statements.

We may start with the example already referred to, of *Stenostoma* (Child, 1904), since here dominance and resorption are very clearly shown. When a solitary Turbellarian is divided, regeneration of a head usually occurs from the anterior cut surface. In *Stenostoma*, however, which is a chain-forming organism, if a cut is made across the body of one of the central zooids, such regeneration from the anterior cut surface does not take place. Instead, the half-zooid which is attached to the anterior end of the posterior half-chain will shrink, assume a more rounded form, and eventually disappear altogether.

Not only this, but the relative age of zooids determines

dominance. In *Stenostoma*, fission occurs according to a regular system, so that the relative age of each head-region in a chain can be determined. If now a cut is made so that a younger zooid is left in front of an older zooid at the anterior end of a piece, this younger zooid, though morphologically complete, will be resorbed by the posterior. If completely isolated from the posterior zooid the younger one would have been capable of leading an independent and normal existence, so that the age-relation of zooids clearly determines dominance.

During the process, 'disintegration' (presumably migration of the cells from the tissues) of the subdermal structures occurs, and the pseudocoel becomes filled with cells and granules. The posterior undestroyed zooid grows more rapidly than usual, apparently because of the excess of nutriment thus provided (although this nutriment is in the pseudocoel and not in the gut). Child did not undertake a histological examination. From his observations *in vivo*, however, it is clear that the cells migrate out of the tissues, as in *Perophora*. The most highly-differentiated organ, the pharynx, disintegrates very early. The intestine, however, does not do so until late. From the ectoderm of the resorbed portion a very gradual migration probably occurs. The portions undergoing resorption are wrinkled and collapsed.

The reversibility of the process is shown by the following observations. If an older posterior zooid has in front of it another almost as old, resorption will begin, but fission will occur before it has finished, and the two zooids will separate; after this the anterior zooid redifferentiates. The converse of this is seen when a long anterior fragment is present. In this case the beginning of regeneration occurs, but reduction finally takes the upper hand, and the whole fragment is resorbed. The rapidity of the change is noteworthy, complete resorption usually occurring in twenty to thirty hours. Provided that the brain-region of an anterior fragment is absent, resorption will occur; even when a system consists of a very long but brainless anterior fragment, and only the brain-region of the posterior zooid, resorption happens.

To sum up, whenever in *Stenostoma* a system is artificially produced in which a posterior brain-region is older than any brain-region anterior to itself, or has a brainless region anterior to it, resorption of such anterior regions will start; it will be completed unless fission of the system occurs during the process, which only happens when an anterior zooid is far-developed.

A brain-region is physiologically dominant over all other tissues of the same and other zooids, and over all younger brain-regions than itself. When a region comes to lie anteriorly to a physiologically dominant region, it cannot maintain itself, and is resorbed. Antagonistic to resorption is the process of regeneration (morphallaxis). Both processes often start simultaneously in a fragment; which of the two eventually gains the upper hand is determined by the age of the fragment. The systems resemble the stolon-zooid systems of *Perophora*, except that the different members of the system are all similar to each other except in age. Further, reversal of the effects by altering the environment has not been attempted. This would provide an interesting field for experiment.

As the facts stand, the dominance is caused entirely by the internal factor of physiological state due to (1) presence and (2) age of brain or brain-region, and resorption is produced when a part is caused to lie in an abnormal position relatively to a dominant region. As Child points out, similar resorption of parts in abnormal positions is frequently seen in grafting experiments in *Hydra* and Planarians. Subordinate portions in a normal position relative to a dominant region do not of course become resorbed.

Once more the essential fact is that, in certain conditions, parts of a system are unable to maintain themselves of their normal size or their normal form, and, once they start dedifferentiating, become subordinate in the system, and can be used as food for the remaining dominant part.

I suspect that investigation would show that the first change, here as in *Perophora*, is the loss of the normal cell-form of the

differentiated organs of the subordinate region, and that resorption follows upon this.

Numerous other cases of tissues, regions, and whole organisms being unable to maintain themselves as such in changed circumstances are known. Of these may be mentioned the degeneration of muscle-fibres when the nerves supplying them are cut. Here the 'normal environment' apparently includes constant nervous stimulation, and in the absence of this the elaborate structure of voluntary muscles cannot be maintained in equilibrium. Similar dedifferentiation of muscle-fibres takes place in the stump of an amphibian limb which has been cut off preparatory to regeneration (Towle, 1901).

In the interesting studies of Child on differential inhibition during development we do not get the total disappearance of one part of the system, but merely a change in the proportions of the various parts. The simplest example studied was the effect of dilute poisons upon the development of the marine Polychaet worm *Chaetopterus* (Child, 1917).

He found that during the earliest stages of development the apical region of the egg and blastula is the most susceptible to various poisons, in certain concentrations a regular death-gradient being obtained from the animal to the vegetative pole. By the time the early trochophore larvae has been produced, however, a new development occurs; the posterior (previously vegetative) region suddenly becomes highly susceptible, its metabolic rate being raised apparently in preparation for the active growth-processes that are about to occur in this region; for the formation of the permanent growth-zone, from which all the body-segments of the adult worm will be produced, takes place here.

The death-gradient will now advance from the two ends of the larvae to meet in the middle region, which, with its lower metabolic activity, survives the effects of the poison longer than the rest. In the later larva the anterior region is differentiated as a head with ciliated band and apical tuft; and posteriorly there is a well-defined growing-region, with a small posterior prolongation.

Immersion of the fertilized egg in solutions of poisons so dilute as to allow development to proceed, while yet exerting an influence on the more susceptible parts of the organism, give the following results. (Essentially similar facts were discovered for other Polychaetes (*Nereis* and *Arenicola*)).

Immersion continuously up to the late larval stage gives a form with both anterior and posterior regions smaller and less differentiated than the normal. The middle region is either almost as large, and of the same form as the normal, or else considerably distended. This latter condition implies possibly that the cells of this region have been able to develop practically normally. The anterior and posterior regions are not so active as normally, and hence are not able to make use of so much of the yolk; there is thus more for the middle region, which is capable of utilizing it, and secretes an excess of fluid. If immersed for eleven hours only, and then replaced in sea-water, the apical region is small, but the growing region as well as the middle region is nearly normal. If, on the other hand, the development is allowed to proceed in sea-water for twelve or twenty-four hours, and the larvae are then placed in the solution, the apical region, having been completed before immersion in the toxic solution, is normal and the posterior end is much affected.

In another paper, giving an account of similar experiments on Echinoderms, he makes an interesting suggestion to account for the great over-development of the skeleton often found in larvae which have grown in dilute solutions of toxic agents. The mesenchyme cells appear to be least susceptible, and thus when the other cells of the organism are inhibited, can obtain a greater quantity of food, which results in a multiplication not only of themselves but of the products of their activity, i. e. the skeleton (Child, 1916).

A recent important attempt to apply similar principles has been made by Robertson and Ray (1920, where reference to earlier papers are given).

Robertson found that mice to whose diet had been added tetelin from the anterior lobe of the pituitary, showed first

a retardation of growth in weight, then an acceleration, and finally lived about 12 per cent. longer than normal controls. Other experiments had led him to conclude that tethelin (or pituitary extract) caused increased growth in cellular tissues, a conclusion strengthened by the recent grafting experiments of Allen (1920) on tadpoles. His explanation of the facts is as follows. Tethelin causes at first an absolute increase in the growth-rate of the cellular tissues of the body; this involves, as we have seen, a relative decrease in the weight of the supporting tissues. Since these latter are the heavy tissues, this involves an absolute decrease in total weight. Eventually, however, the characteristic relation between the amounts of cellular and supporting tissues is established, but later than normal. Relative increase of the supporting tissues characterizes old age; and the onset of senility is delayed by that period by which the establishment of the cellular-supporting balance was postponed. The reason for the more rapid growth of the cellular tissues at the beginning is that the tethelin stimulates them to greater activity, and that consequently they obtain first call on the available foodstuffs.

This view-point, it will be seen, is very similar to that of Child.

A beautiful example of differential inhibition depending only on the two quantitative factors of size and distance is given in the interesting paper of Detwiler (1920; see especially pp. 149-51). Detwiler transplanted the limb-rudiments of *Amblystoma* autoplastically, cutting the rudiments out and transplanting them a varying number of segments posteriorly from their normal position. The experiments were undertaken at a stage when the rudiments were represented only by circular thickenings of somatopleuric mesoderm in segments 3-5. He found, as had previous workers such as Harrison, that in many cases the rudiment was not completely excised, a few of its cells being left in the normal position. When this was so, these cells usually begin to regenerate on their own account. It is of interest to note that this regeneration is

greater when the wound is not covered—a result presumably due to the greater stimulation which the unexercised limb-cells then receive (Harrison, 1915).

After a short time a small nodule of cells begins to protrude from the body in this region. If the main limb-rudiment is completely removed the nodule may grow into a perfect limb. When, however, the main limb-rudiment is transplanted less than four segments back on the same side, these nodules, after growing a longer or shorter time, begin to shrink, and eventually disappear altogether. When the limb-rudiment was only transplanted one segment back the nodules appeared after about four days, but very speedily began to decrease and had disappeared after eight days. When the limb was transplanted two segments back the nodules continued to increase till the fifth or sixth day, and had disappeared by the eleventh day; when the distance of transplantation was three segments, nodule-growth continued until the tenth or eleventh day, when the 'nodule' was almost as large as the transplanted limb; but after this, decrease set in, and all nodules eventually disappeared, although not until the eighteenth to twentieth day. Finally, when the main limb-bud was removed more than three segments from its original site, the regenerating nodules always developed into a normal appendage, so that two limbs were produced from the one original rudiment.

The cells of the limb-bud constitute an equipotential system, as Harrison has shown. It is therefore clear that the inhibiting effect exerted by the main transplanted rudiment on the cells left at the original site must be due simply to the greater size of the former. The strength of this 'dominance', however, also depends upon the distance of the two systems; and when this distance is increased beyond a certain limit, there is no longer any inhibitory effect. If we like, we may say that the reason why the cells constituting the normal limb-rudiment of *Amblystoma* do not usually form more than one limb is that they occupy such a small area that any one rudiment growing within that area inhibits the growth of any other.

Detwiler did not investigate the actual mechanism by which

the 'nodules' decreased in size, and leaves it open as to whether the cells composing them are actually translocated into the main limb-bud, or are simply resorbed into the body. The former view is less probable on general grounds, and the latter is supported by the facts of resorption in *Perophora*. The limitation of physiological dominance by distance has already been brought out by Child (1915 *a*, chap. 5), but is here particularly well illustrated. The relation of dominance to simple size-difference between two portions of otherwise identical tissue has not, however, so far as I am aware, received any special attention, but is obviously of considerable theoretical importance. Further, in no other case with which I am familiar, is the importance of purely quantitative relations so well brought out. It is perfectly clear that inhibition and consequent resorption can take place at any stage of growth of the 'nodule' (regenerating limb-rudiment), and that it is not due to anything in the nodule itself, but entirely to its relations with a second developing system.

We now pass to the very different field of neurology and psychology.

In recent years the phenomenon known as mental regression has been carefully studied. Patients suffering from this return to an earlier stage of mental existence. Grown men may show the behaviour and the mental processes of boys of ten or five or even younger. A review of our knowledge of this condition is given by Nichol (1920).

When properly analysed this state of affairs would seem definitely to be due to the presence, in individuals affected by it, of two competing systems of mental organization, i. e. of two possible main channels for the flow of 'nervous energy'. (I purposely use this latter somewhat vague but non-committal term to emphasize the fact that the existence of competing systems and of some form of activity transmissible along their paths is all that we need to assume for a preliminary discussion of the problem.) In normal conditions the adult system is dominant, the main flow of nervous energy is along its paths, and the childish system or systems are dormant, existing for the

most part only as potentialities of action. Under severe stress (e. g. modern warfare, prolonged worry, &c.), the adult system becomes in some way affected. It is no longer so easy for the nervous energy to flow along its paths. Under these conditions there is more nervous energy available for the other, juvenile, system, which has remained undamaged. Finally, there will come a moment at which the balance is so altered that the adult system ceases to be dominant, and the potentiality of the juvenile system is transformed into actuality. The juvenile system now becomes dominant in its turn, and the adult system retreats into potentiality. During recovery a remarkable picture is presented: the two systems are almost equally balanced, and we get—not a blending of the effects of both—but a rapid alternation, first one and then the other, the two never co-existing. A somewhat similar state of affairs exists in *Perophora*; once absorption of either portion has started it proceeds rapidly. Alternation, however, is not possible, since in *Perophora* it is structure, and not merely possibility of function, that is being destroyed.

In the neurological cases structure is not destroyed. Further, the rapidity of change from the dominance of one system to that of the other is enormously more rapid, since this is apparently accomplished simply by the passing of a threshold-value. Once this is passed a sluice is opened, and a different neural system flooded so as to permit of function. For this sudden appearance of one or the other sub-system some psychotherapeutic writers use the expressive term 'puffing-up'. It is a well-known phenomenon of convalescence in such cases.

Such occurrences are one aspect of the general principle laid down by Hughlings Jackson, that, as the result of lesion, 'dissolution occurs first in the most highly-organized products of neural or mental activity, leaving the more lowly at liberty to express themselves freely in the resulting symptoms'. This, however, only stresses the aspect of differential inhibition, not that, of equal importance, of intra-organismal struggle.

Part of this latter aspect of the question is expressed, however, by Head (1918), who lays down as one of his general principles of neurology that 'Integration of function within

the nervous system is based on a struggle for expression between many potentially-different activities. Integration of function, however, is not all. A number of integrated minor systems may exist, one in actuality, the rest in potentiality, in the developed human psycho-neural system as a whole; and there is also a form of struggle between them. The particular type of mental disorder known as regression is only one special case of the results of differential susceptibility among two or more such minor systems. In other so-called neurasthenic cases the second, normally-suppressed system may not be a system of childish memories, but an imaginary 'ideal' world of thought along whose paths consciousness flows instead of along those necessary to maintain adaptation to everyday life; or else it may be the system of 'negative' emotions, leading to depression and possibly to suicidal attempts. Dissociation of personality and subsequent alternation of the sub-personalities may also, though less directly, be included under the same rubric. Rivers, in a recent work (1920), has emphasized the same point of view; he points out for psychological systems what I have drawn attention to in this paper for physical systems—that reversal of dominance in a balanced system may occur either through the action of unfavourable agencies on the dominant system (differential inhibition) or of favourable agencies on the subordinate system (differential stimulation).

In a case of regression mentioned by Dr. W. MacDougall and Dr. Hadfield in their lectures and confirmed to me in conversation by Lt.-Col. Good, of Ashhurst Hospital, a young man actually regressed to the condition of an infant.¹ He was unable to talk or walk, and could tolerate no food except milk. (By some freak of the nervous mechanism two associations and two only remained from adult life: if a cigarette were offered him he would light and smoke it; when shown a horse or a picture of a horse, he would get astride of some object and 'tehk' as if encouraging a horse. It turned out that he

¹ Since the above was written, I find that an account of this and similar cases has been published by MacDougall in 'Journ. Abn. Psych.' 15, 1920, p. 136.

had been a jockey.) His recovery was interesting for various reasons. The intolerance for all diets save milk he lost earlier than the other infantile symptoms. As regards purely mental symptoms his growth or redifferentiation was gradual and progressive, though with considerable rapid oscillations. It is therefore clear that the picture is not quite as simple as I have drawn it above. Each stage is really in some ways dominant to the one below, subordinate to the one above, and if there has been a considerable degree of regression, the redifferentiation must apparently be by steps (although the regression itself is a sudden instantaneous process). In the normal adult each lower stage is kept in its proper place in the hierarchy, and most of the associations and types of reactions connected with it exist in *potestate* only. When it is released from the inhibitory control of the processes associated with higher stages it becomes dominant, and then these potential associations, memories, and reactions become actual and functional again. Normally, since each stage of growth represents a necessary step towards the next stage, some of the reactions of each stage are functional even in the adult, as foundations for normal adult activity; but they are altered by the dominant higher processes to a form different from that which they would have if released from control. This is parallel, though not identical, with the behaviour of dominant and subordinate regions in regeneration (see later). Regression takes place suddenly to that stage whose system has been encouraged; if the patient has dwelt upon a particular time of childhood, to the system associated with that time; if he has dwelt on mere release from control, to an infantile stage. But recovery must be by gradual building-up, as in physical development.

Individual mental development is thus an epigenetic process; and the different stages of this development are arranged in a functional hierarchy or series in which each stage is dominant to the one below, subordinate to the one above.¹

¹ The alternation of dominance seen in dual and multiple personality (Prince, 1908, 1920) is presumably based upon essentially the same principles, the difference being that typically the two systems are very evenly balanced,

We shall now see that similar relations may exist in non-conscious neural processes, of which the lower have never been fully dominant in ontogeny (though possibly in phylogeny).

This is well shown by the observations of Head and Riddoch (1917) on the activities of 'spinal man'. They found that when the spinal cord was completely divided, the reflex activities which manifested themselves after the initial shock-period were very different from those occurring in the uninjured individual. In the normal person the activities of the spinal cord are modified by influences reaching it from pre-spinal levels. The isolated spinal cord, however, responds to stimulation predominantly by a type of 'mass-reflex' not normally seen in man. In 'spinal man' any form of nocuous stimulation to a hind-limb causes not merely flexion of the limb stimulated, but violent flexion of both limbs, abdominal contraction, voiding of the contents of the bladder if the contained fluid is above a certain very small volume, and sweating. Conversely, injection of the bladder with fluid induces a flexor spasm of the lower limbs, combined with sweating. (The reaction may be called an excessive and non-discriminate reaction to harmful stimuli, resembling in many ways that seen in certain lower animals, e.g. the toad, in which voiding of the bladder accompanies limb-flexion when the animal is alarmed by handling.) The same mass-reflex also appears in higher forms and in man himself when the higher centres are put out of action under the influence of an excessive degree of an emotion such as fear (differential inhibition). The mass-reflex may be looked on as a very primitive response of the organism to nocuous stimuli.

In higher forms the mass-reflex has become subordinated to the influence of other types of reaction; among these are the postural reactions and the conscious direction of movements of escape. Head and Riddoch found that so long as any

and both adapted (though incompletely) to adult life. The emergence of the juvenile personality 'Sally' in Morton Prince's case is especially interesting as it only occurred when the normal control was impaired through the dissociation of the adult personality into two.

remains of postural control were present in their patients—which indicated that some connexion was still present with pre-spinal centres—the mass-reflex did not appear. In other words, in the course of phylogenetic evolution, a compound mechanism has been evolved, the parts of which stand to each other in a relation of dominance and subordination. But here the dominance appears to be only slightly reversible, as opposed to the cases of *Perophora* and of mental regression. Here the subordinate system is so thoroughly under the control of the other (presumably owing to certain structural relations and to innate physico-chemical peculiarities inherent in synapses concerned with inhibition), that it is apparently impossible to tilt the balance so as to make the subordinate system the dominant one for long together, so long as both are in organic connexion. It is only when the two systems are separated from each other that the real nature of the subordinate system can be studied as it exists apart from controlling influence from without. As indicated above, differential inhibition through fear may induce a short temporary reversal of dominance.¹

Child has pointed out that a somewhat similar (and also simpler) relation subsists between the dominant and the subordinate regions in many low forms of animals, such for example as Planarians. Here, so long as the head region is exerting its dominant or controlling influence, other portions of the organism cannot form a head. But when this influence is removed, either by the amputation of the head or by the 'physiological isolation' of parts of the organism (by their removal, through growth, beyond the radius of influence of the head), then the most anterior part of the isolated region at once reacts by producing a head (Child, 1915*b*, p. 96 et seq.). In Head's spinal case, however, after isolation the subordinate system does not take on the characters of the dominant system, but assumes a form which is peculiar to itself.

¹ The views of Head and Riddoch have been recently criticized (e.g. 'Medical Science', vol. 4, 1921, pp. 141, 430). The fact of decerebrate rigidity, however, would, among others, equally well serve to illustrate the principle of neurological dominance and subordination, although here we remain without phylogenetic analogies.

We may now leave the nervous system and return to physiology. As an example in mammals, and one concerned only with the parts of one organ, the following will serve.

As is common knowledge, the testis in mammals consists of several functionally-distinct parts. Apart from blood-vessels and nerves there are (1) the germ-cells (spermatogonia, spermatocytes, spermatids, and spermatozoa), (2) the cells of Sertoli, (3) the interstitial cells or cells of Leydig, (4) connective-tissue cells. In the normal testis these exist in proportions which do not vary beyond narrow limits. Various agencies, however, will upset this balance. The germ-cells are the most susceptible. Exposure of the testis region to X-rays or to Mesothorium; or ligature or section of the vas deferens; or abnormal position in the organism, which can come about spontaneously as in natural cryptorchism or can be produced experimentally as in artificial cryptorchism or by transplantation, will bring about some degree of degeneration of the germ-cells. This is accompanied in every case by a hypertrophy of the interstitial cells. The cells of Sertoli are usually unaffected. It would appear that these latter are not cells capable of rapid multiplication. The chief competition is therefore between the germ-cells and the interstitial cells. The former are in some way dominant; when they are damaged, a check on the latter is removed, and their active increase results. Whereas removal of the testis to an abnormal environment usually results in the permanent disappearance of the germ-cells, X-ray treatment, if not very intense, only damages them temporarily. Later they regenerate, and finally come to have their old proportion once more. The increase in the number of interstitial cells only lasts until this regeneration starts, and is followed by a decrease. Finally, the normal equilibrium is re-attained.¹

¹ See also R. Goldschmidt, 'Biol. Centralbl.', 36, 1916. p. 160. In Lepidopteran testes cultivated in tissue-culture, normal spermatogenesis occurs. But the germ-cells always die before the cells of the follicle. When this happens, the follicle-cells, which have till then remained normal, start at once to multiply at a rapid rate.

The germ-cells are thus, in normal circumstances, partially dominant over the interstitial cells, and are also more susceptible than they are. This is the same relation that we found to hold good between the zooid and stolon of *Perophora*. Furthermore, it appears that in the testis a similar relation is to be found between the interstitial cells in their turn and the connective tissue (and Sertoli cells). Transplanted testes, as we have said, first lose their germ-cells and show increase of interstitial tissue. Within a few months the Sertoli cells also degenerate and disappear (Steinach, Sand). We may take this to mean that these cells, while not increasing after the loss of the germ-cells because they are not a multiplicative type of cell, are slightly less resistant than the interstitial cells. Even these, however, are less resistant to unfavourable conditions than the connective tissue. After a longer or shorter period (usually several months) in the abnormal situation, the interstitial cells in their turn start to decrease in number, and now it is the connective-tissue cells which show a corresponding increase. Finally, the 'testis' comes to consist of nothing but connective tissue and blood-vessels. This is also seen in some few cases of cryptorchism.

We have thus a system in which there enter four variable sub-systems. One of these, for a reason which we can conjecture but not prove, does not increase when others decrease. The other three, however, are all in that state of dynamic equilibrium which we have seen in its simplest manifestation in *Perophora*. But this time they are arranged in a series, A being physiologically dominant over B, and B in its turn over C. Normally, therefore, the relative proportions of the three tissues are regulated according to the activity of A. When A is adversely affected B increases, but not C. C, however, increases when both A and B have been affected.

If such a type of system were to exist, it should follow that in some (abnormal) circumstances somewhat different conditions should obtain, and that a slightly different end-result should be brought about. As a matter of fact, in some of the transplantations of Sand, this did occur. In three cases both

germ-cells and interstitial cells disappeared, leaving only Sertoli cells and connective tissue. In one other case the germ-cells and Sertoli cells were much less affected than the interstitial tissue. This recalls the varying behaviour of the stolon-zooid system in *Perophora* according to the internal condition of the zooid. (See Lipschütz, 1919, Chap. IV, where full references are given.) Another view of an almost identical problem is given by the varying response of the mammalian ovary to different intensities of X-ray treatment (Lipschütz, 1919, Chap. V, p. 205).

The conclusions we reached in discussing Detwiler's results (pp. 679-687) are of importance when we come to apply the principles of dominance, differential inhibition, and resorption to an explanation of the phenomena of metamorphosis. In metamorphosis, as I have pointed out elsewhere (Huxley, 1921 *b*), we have to think of the full-grown larva as consisting of two minor systems in competition with each other—the differentiated system of larval organs, and the developing system of adult organs. The two enter into a state of balance. This balance may be tilted in favour of the adult, or kept at the existing tilt which favours the larval system. It has often been maintained that the time of metamorphosis was determined by the production of a given relative quantity of some definite substance within the organism, e.g. thyroid secretion in the larvae of *Amphibia*. Such a concentration of a particular substance is often the effective agent in tilting the balance, but it is not the essential cause of metamorphosis. The essential cause of metamorphosis is that two mutually incompatible systems are in a state of dynamic physiological equilibrium within the same organism.

In Echinoderm metamorphosis the mechanism for upsetting the balance appears to be simpler than in *Amphibia*. Experiments of Runnström (1917) and of my own, an account of which is now in the press, indicate that exposure of the pluteus tissues to unfavourable agencies of various descriptions will lead to their dedifferentiation and partial resorption. In nature the actual chain of events leading to this result appears to be as

follows: the Echinus rudiment at the start grows concomitantly with the Pluteus. After a certain time, however, it becomes so large that its weight drags the larva to the bottom. Here the conditions, as regards both food and general environment, are unfavourable to the pluteus tissues; these begin to dedifferentiate, and as soon as they have passed a certain critical stage in the process the Echinus tissues become dominant and are able to develop further at the expense of the larval organization. In the broadest terms the balance in Amphibia is regulated mainly from within, in Echinoids mainly from without; but in both cases the possibility of the sudden change which we call metamorphosis depends on the co-existence of two systems in the same organism which are very closely balanced as regards physiological dominance.

To sum up, we may say that the facts of physiological dominance of inhibition of growth, of resorption, and of the state of balance which exists among the parts of any organism and is the dynamic expression of Roux's 'Kampf der Teile', are all intimately connected. As a matter of fact physiological dominance is rendered most obvious when it can be reversed, as in Perophora or in metamorphosis—and that is when the balance between sub-systems is very close.

The various examples discussed may perhaps be made clearer by the use of symbols. In every case let A = a dominant system; B a system normally subordinate to A; C one normally subordinate to B and also to A. An arrow \downarrow indicates dominance, pointing towards the subordinate system. Brackets () indicate subordinate condition. Dashes (A', B', &c.) indicate alteration of the system from its original condition to another. Erasure (~~A~~, ~~B~~, &c.) indicates disappearance of a system by resorption. Suffixes (A₁, B₂, &c.) indicate homologous systems in order of age or size. Enclosure

$\left(\boxed{A}, \boxed{\begin{matrix} A \\ B \end{matrix}} \right)$ indicates passage to a non-functional state.

Plus sign (A+, B+, &c.) indicates increase of the system.

1. *Clavellina*. A = zooid, B = stolon.

(a) Normal.



(b) Reduced.



2. *Perophora*. A = zooid, B = stolon.

(a) Normal.



(b) Starved, water changed.

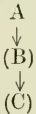


(c) Starved, water not changed.



3. *Planaria* or single *Stenostoma* zooid. A = brain-region, B = pharynx-region, C = tail-region.

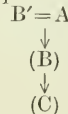
(a) Normal.



(b) After decapitation.

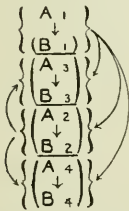


(c) Subsequent regeneration.



4. *Stenostoma* chain. A = brain-region. B = rest of zooid. A₁ B₁ = oldest, A₄ B₄ = youngest zooid.

(a) Normal.



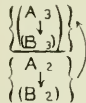
(b) Transection giving brain-region behind posterior brainless region of another zooid.



(c) End-result from (b).



(d) Transection giving older zooid posterior to younger.



(e) Result of (d).



5. 'Spinal man.' A = cerebral centres, B = mass-reflex.

(a) Normal.

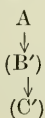


(b) After transection of cord.

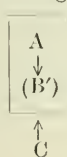


6. Mental regression. A = adult system, B = juvenile system, C = infantile system. (Only three systems given for simplicity's sake.)

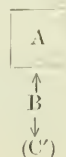
(a) Normal.



(b) After regression.



(c) During recovery.



7. Testis. A = germ-cells, B = interstitial tissue, C = Connective tissue.

(a) Normal.	(b) Transplanted, after short period.	(c) Ditto, after longer period.
A	A	A
↓	B+	B
(B)	↓	C++
↓	(C+)	
(C)		

8. *Amblystoma* limb-buds. A_2 = transplanted limb-bud, A_1 = regenerated remains of limb-bud in original position.

(a) After transplantation to a distance of more than four segments.	(b) End-result from (a).	(c) After transplantation to a distance of less than four segments.	(d) End-result from (c).
A_2	A'_2	A_2	A'_2
A_1	A'_1	↓ (A_1)	A'_1

9. Metamorphosis of Echinoids. A = larval tissues, B = adult tissues.

(a) At the time when larva sinks to bottom.	(b) Shortly after.	(c) End-result.
A	(A')	A
↑↓	↑	B'
B	B	

9. SUMMARY.

1. The social Ascidian *Perophora viridis* may differentiate in either of two distinct ways, or by a mixed method: (a) by reduction to a spheroidal mass, as in *Clavellina*; (b) by incipient reduction as in (a), but followed by total resorption into the stolon, which may grow during the process.

2. Resorption is due to the migration of the individual cells out of the tissues into the haemocoel.

3. In certain conditions the zooid maintains itself, in spite of food not being provided, at its original size and in perfect health. This it does by resorbing the stolon.

4. Experiments with dilute solutions of KCN show that resorption of the zooid occurs in slightly unfavourable conditions, which affect the sensitive zooid more than the less highly-organized stolon.

5. The results are to be explained as follows: (a) In the competition between zooid and stolon the zooid normally is dominant because metabolic processes take place at a greater rate in it than in the stolon. The stolon is therefore starved at the expense of the zooid. (b) The zooid is more susceptible than the stolon to toxic agencies. (c) In low concentrations of such agencies it is therefore affected while the stolon is not. (d) As a result it begins to dedifferentiate. Dedifferentiation is here accompanied by the migration of the cells out of the tissues. (e) The speed of its metabolic processes is now no longer greater than that of the stolon's. It is therefore now starved at the expense of the stolon. (f) Any cells migrating out of the tissues are removed by the normal circulation, by the stolon-circulation (irregular pulsation of the stolon), or by utilization as food by the stolon. As in chemical reactions where the end-products are removed, the reaction thus runs to its limit, i. e. to complete resorption of the zooid.

6. Stopping the circulation by means of KCl results in dedifferentiation accompanied by a much smaller degree of resorption.

7. At low temperatures (about 5° C.) some dedifferentiation occurs; but there is very little resorption, apparently owing to the cessation or slowing of the heart-beat.

8. Partial dedifferentiation is recorded in *Amaroucium* and *Botryllus*.

9. The significance for general biological problems of dominance due to high rate of metabolism, of differential susceptibility and of dedifferentiation, is discussed.

10. The similarity of certain psychological and neurological phenomena is noted (mental regression, alteration of spinal reflexes when freed from cerebral control, &c.).

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ILLUSTRATIONS.

All figures are drawn to scale with the Abbé Camera lucida. Otherwise they are semi-diagrammatic. All were drawn at table level; the magnification is indicated for each figure.

Fig. 1.—Clavellina type of reduction ($\times 25$). Two zooids, A and B, isolated without stolons. *a*. Day of operation. B has a trace of stolon-connexion. *b*. After forty-eight hours. Reduction started earlier in B. Both have formed short stolons, but that of B remains within the test. A has only just started to reduce. *c*. Advanced reduction (three days for B, four days for A). The stolon of B is large and lobulated, but has not emerged from the test. B is spheroidal and opaque, in stage 4-5. A is in stage 3, which it did not reach till after three days. Its stolon has grown, and is distended with cells. A's heart was beating slowly, B's had almost stopped.

Figs. 2 and 3.—Growth or maintenance of zooid at expense of stolon.

Fig. 2 ($\times 80$).—*a.* Immature zooid on day of operation. *b.* The same, perfect, after three days. The stolon has been much reduced both in length and breadth. A small bud had formed and been absorbed. *c.* After five days. Further reduction of the right end of stolon; zooid in first stage of reduction, which has led to a slight dilatation of the left end of the stolon. *d.* Stolon-tip from a similar system after three days, showing shrunken appearance.

Fig. 3 ($\times 25$).—*a.* A system on the day of operation. *b.* The same three days later. Zooid actively functional, stolon much drained in all dimensions.

Fig. 4.—Maintenance of bud following resorption of first zooid. *a.* ($\times 80$). Original zooid in stage 4 of reduction, after two days. Stolon healthy. *b.* ($\times 80$). After three days. Stage 4, but smaller; meanwhile a bud had formed to the left of the zooid, and by now was 50 per cent. larger in diameter than the zooid. *c.* ($\times 40$). After five days the zooid had disappeared. The remains of its test is seen. The right part of the stolon, to the right of the bud, has also been resorbed, and resorption is beginning in the other portion, as shown by the extent of its test. *d.*, *e.* ($\times 40$). After seven days. In *d.* the tip of the stolon is shown contracted, in *e.* expanded with blood. *f.* After twelve days. The stolon has almost disappeared. The zooid is practically unchanged in size or development.

Figs. 5–15.—Resorption of zooids and growth of stolons.

Fig. 5 ($\times 25$).—Stolon-growth. *a.* A system on the day of operation. *b.* The same, but stolon only; four days later. The zooid was in stage 2 of reduction. A bud is seen on one stolon-branch. Note the test bridging concavities of the stolon.

Fig. 6 ($\times 40$).—Early stages of resorption. *a.* After two days. Zooid just reaching stage 3. The exhalant siphon is still slightly attached to the test. Faint traces of gills visible. The stolon-branch *B* and the tip of *A* represent new growth. *b.* Eight and a half hours later. The zooid is in stage 3–4, and has shrunk considerably; *B* has grown.

Fig. 7 ($\times 64$).—Zooid in stage 3–4 of reduction, showing heart and traces of inhalant siphon and stomach; note the double stolon-connexion.

Fig. 8 ($\times 64$).—Zooid in stage 4 of reduction. The heart is seen end on.

Fig. 9 ($\times 64$).—Later stages of reduction. *a.* Zooid in stage 4, after two days; ectoderm in places eubial. A stolon outgrowth had occurred. *b.* The same, ten hours later (test omitted); further shrinkage. Ectoderm all cuboidal. *c.* Fourteen hours later (test omitted); further shrinkage. A new stolon outgrowth has occurred. *d.* Forty-eight hours later (five days in all). It is now in stage 6 (after four days it had reached stage 5). The pale ovoid is probably the remains of the stomach. Note the slight reduction of the test. *e.* Twenty-four hours later (six days). Zooid portion smaller than stolon-connexion.

Fig. 10 ($\times 64$).—Zooïd reaching stage 5. The stolon was attached to another zooïd, and showed active circulation. It was hard to be sure whether the heart was beating.

Fig. 11 ($\times 100$).—Zooïd in stage 5. Stolon as in 10. Zooïd ectoderm cuboidal. Solid organ-remains fill most of the zooïd.

Fig. 12 ($\times 64$).—*a.* Zooïd in stage 4, after two days. *b.* The same, nine and a half hours later (from a different aspect). Stolon as in 10.

Fig. 13 ($\times 64$).—Zooïd in stage 5 of reduction. Zooïd of the same opacity as the stolon.

Fig. 14 ($\times 64$).—Zooïd in stage 5-6. Opacity as in 13.

Fig. 15 ($\times 64$).—Zooïd in stage 6. Some remains of organs visible.

Figs. 16-18.—Reduction at low temperature.

Fig. 16 ($\times 32$).—After eight days; early stage of reduction. Note considerable opacity combined with open siphons. Heart beating, but circulation only in right half of the stolon-connexion. Note a new stolonie outgrowth into the test of the zooïd.

Fig. 17 ($\times 64$).—A similar zooïd after eight days. Débris on siphons, which are open. Heart not beating, but visible.

Fig. 18 ($\times 64$).—Similar; but a slightly later stage of reduction. Depressions still mark the siphons. Heart beating, but very faintly.

Figs. 19-20.—Reduction in KCl solutions.

Fig. 19 ($\times 25$) (50 c.c. sea-water+4 c.c. $m/2$ KCl).—*a.* Early stage of reduction, after one day. Inhalant siphon-lobes of test separate from siphons. Outgrowths at the end of stolon. *b.* The same, twenty-four hours later. Cell-strands attach siphon-regions to test. No sign of internal organs. Stolon healthy.

Fig. 20 ($\times 25$) (50 c.c. sea-water+8 c.c. $m/2$ KCl). Similar to 19, *b.*, except that the stolon as well as the zooïd has been adversely affected (shrinkage, cuboidal epithelium).

Figs. 21-3.—Reduction in KCN solutions.

Fig. 21 ($\times 25$).—In $m/2,000$ KCN. *a.* Before treatment. The zooïd is a not quite developed bud. *b.* After twenty-four hours. Zooïd in stage 3 of reduction. Stolon slightly shrunk, but crowded with cells, and with attempts at new growth.

Fig. 22 ($\times 25$).—In $m/4,000$ KCN. *a.* Before treatment. *b.* After forty-eight hours. Zooïd much reduced. Stolon crowded with cells, but shrunken; no new growth.

Fig. 23 ($\times 25$).—In $m/32,000$ KCN. *a.* Before treatment. Zooïd a not quite developed bud. *b.* After twenty-four hours. Zooïd considerably reduced, stolon with clubbed ends and with new growth (within test only). *c.* In reversed position after forty-eight hours. Zooïd much reduced. Stolon crowded with cells, and with new growth outside test.

Fig. 24 ($\times 340$).—To show pulsation of stolon. The same stolon-tip (*a*) expanded; (*b*) (less than a minute later), contracted. The position of

the test (x) did not change, and a space was left between it and the ectoderm when contraction occurred. Note the thickened epithelium in contraction, with irregular outline externally. Note the small outgrowth in the expanded state; this was not observed after contraction. The stolon remained for a few minutes contracted, then expanded in under a minute; and vice versa. The blood-cells are not figured.

Fig. 25 ($\times 340$).—A large lateral outgrowth, on the same stolon as that shown in fig. 24. The blood-cells are shown in the outgrowth itself, but only a few indicated elsewhere.

Fig. 26 ($\times 340$).—A normal growing stolon-tip. Note the columnar epithelium at the extreme tip. Close to the tip there are very few green blood-cells, the majority being white. Then comes a zone where a considerable proportion are green, and then one where they are in the majority. The circulation, though active, did not extend into the densely-packed region drawn.

Figs. 27 and 28.—Dedifferentiation in *Amaroucium*.

Fig. 27 ($\times 80$).—A young oozite in stage 2-3 of reduction. The dense anterior mass was orange-red. Portions of the intestine are seen below. Muscular contraction of the whole organism still took place at intervals.

Fig. 28 ($\times 80$).—A blastozooite dedifferentiated in weak alcohol, stage 3. Note the cell-masses outside the main body of the organism.